EFFECTS OF PROPYLENE GLYCOL 1,2-DINITRATE ON RHESUS MONKEY VISUAL EVOKED RESPONSE AND SIDMAN AVOIDANCE TASK

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EFFECTS OF PROPYLENE GLYCOL 1,2-DINITRATE ON RHESUS MONKEY [illegible] AND SHOCK-AVOIDANCE TASK

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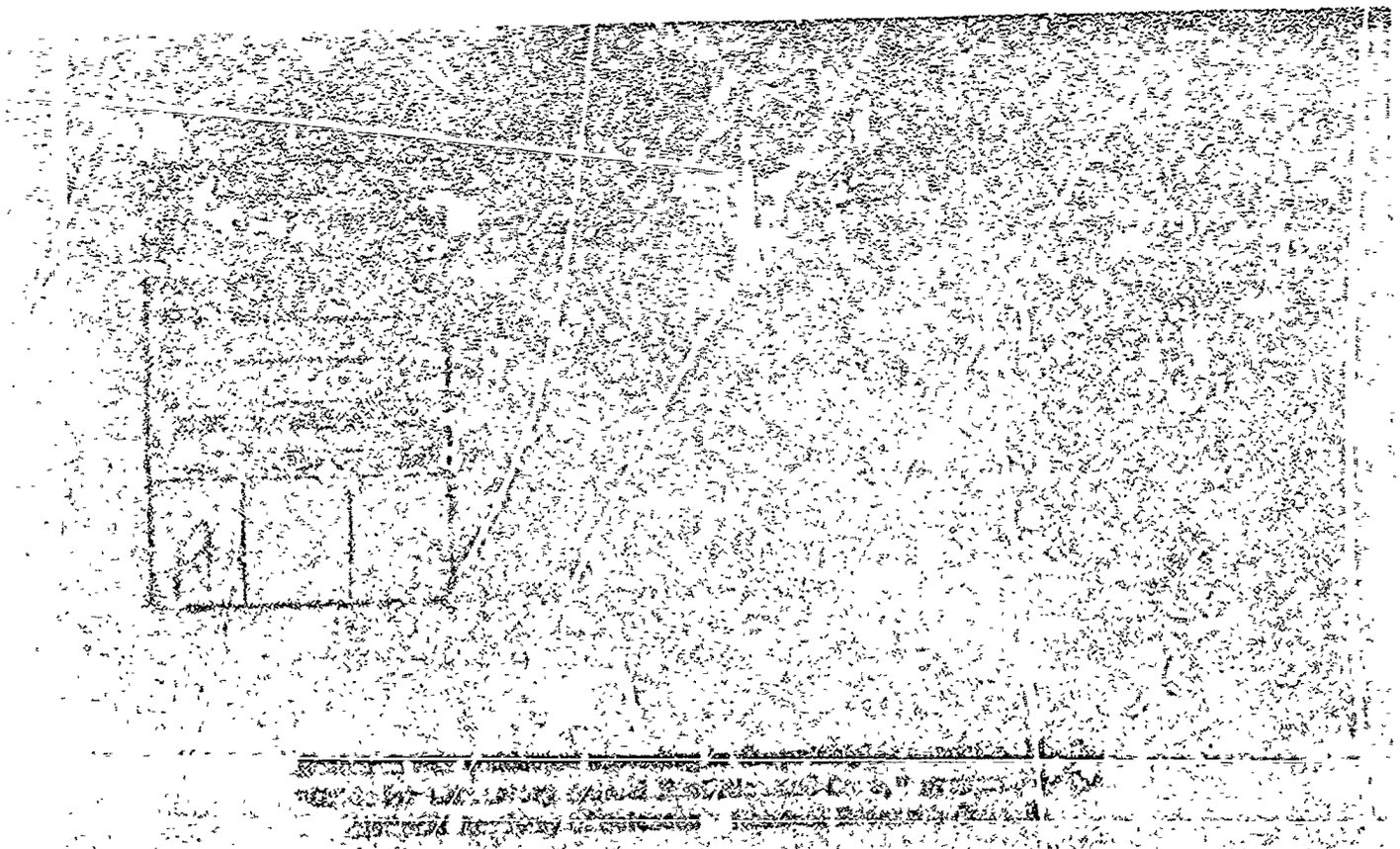

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ABSTRACT

Two rhesus monkeys (Macaca mulatta) were exposed to propylene glycol 1,2-dinitrate (PGDN) vapors for 4 hours at concentrations ranging from 2 ppm to 33 ppm. Visual evoked response and Sidman avoidance task data were collected after 2 hours and 4 hours of exposure. Performance on the Sidman avoidance task was not affected at any dose level. The only component of the visual evoked response which PGDN affected was the late positive (100-150 msec) wave, which increased 20 percent at 2 ppm and decreased 25 percent at concentrations up to 33 ppm. It was possible to reverse the effect at 33 ppm by altering tension on the monkey's Sidman response lever; this raises the question of specificity of visual evoked response to PGDN. Because of the reversibility of the effect, and since the changes occurred at a latency and amplitude consistent with those expected from the aversive, distracting properties of the vapor, the visual evoked response changes could not be unambiguously attributed to neurotoxic changes in the central nervous system.

I. INTRODUCTION

Jones et al.[8] reported on a series of propylene glycol 1,2-dinitrate (PGDN) vapor experiments, including several on nonhuman primates. In summary: (1) Rhesus monkeys exposed to 73 to 102 ppm for 6 hours showed vomiting, pallor, cold extremities, semiconsciousness and clonic convulsions. (2) A squirrel monkey died after exposure to 61 ppm for 3 days. (3) Nine squirrel monkeys were exposed for 90 days to 10, 16, or 35 ppm PGDN vapors; one died after 31 days at the 35 ppm level. No other deaths occurred, although there were fatty changes in the livers and hemosiderin deposition in livers and kidneys of the other eight animals. (4) Rhesus monkeys, exposed to 39 ppm for 90 days, had no significant changes in visual discrimination or visual acuity threshold tests. Young et al.[20] reported no significant changes in cued or free operant (Sidman) avoidance behavior of two rhesus monkeys exposed to levels of PGDN vapors which were gradually increased from 0.3 ppm to 1.3 ppm over a period of 125 days. These data indicate a fairly high degree of tolerance of monkeys to PGDN vapors.

However, Stewart et al.[18] reported that, in human subjects at 0.2 ppm and above, PGDN vapors produced headache and alteration of the visual evoked response (VER). At 0.5 ppm, impairment of balance became manifest. Exposures above 1.5 ppm were not attempted.

The wide disparity between the human and monkey data may be due, in part, to lack of comparability of the criteria selected or the tasks performed in the different experiments. In an attempt to explain these differences, the effect of PGDN on VER and Sidman avoidance task was studied in monkeys.

II. METHODS

Two chair-restrained male rhesus monkeys (Macaca mulatta) weighing 8 and 10 kg were exposed on several occasions to PGDN vapors at 2 to 33 ppm. The 10-kg monkey was exposed to 2 ppm three times, and once at 7 and 20 ppm. The 8-kg monkey was exposed to 3, 10 and 33 ppm. There was a minimum of 1 week between exposures.

The Rochester-type inhalation exposure chamber was approximately 2 m^3 in volume and was modified for continuous use.[5] A strobe photo stimulator was located outside the chamber and behind the monkey. Chamber windows were covered with white cardboard to eliminate visual distraction of the monkey, and to provide a diffuse reflective surface so that the light from the strobe would be uniformly distributed throughout the chamber, reducing VER variability from changes in light intensity and direction of the animal's gaze. Air was passed through a gas washing bottle containing PGDN, delivering PGDN vapor into the chamber. To maintain desired vapor concentrations, dilution air was passed through the chamber at 0.5 to 1.0 m^3/min. The chamber atmosphere was monitored chromatographically. A sample of chamber atmosphere was drawn by vacuum through a seven-part automatic switching valve into a calibrated sample loop and then to a chromatograph equipped with a 6 ft x 1/4 in. glass column containing 2.92 percent OV-17 on Anakrom Q 70/80 mesh. The system operated at a temperature of 110°C using nitrogen at 70 ml/min and an electron capture detector at 150°C with a voltage of 20 V dc. Long lines and switching valves were heated to prevent condensation. A second, independent method of analysis was used by drawing a known volume of the atmosphere through a bubbler equipped with a coarse frit and

containing ethyl alcohol as the absorbing media. The sample was then read at 200 nm on a spectrophotometer.

Each experiment lasted approximately 6 hours. During the 1st hour VER and performance data at zero PGDN concentration were collected. Vapor was then introduced into the inhalation chamber, reaching the desired concentration in 30 minutes to 1 hour. VER and performance data were collected at 1.5 hours and 3.5 hours after the desired PGDN concentration was reached. Each data collection period lasted 1 hour, and were called D_0, D_2, and D_4 respectively (data at 0 hour exposure, 2 hours exposure, 4 hours exposure). Data were obtained the day before the PGDN exposure to control for drift during the experiment and to determine day-to-day stability.

The EEG was recorded from a single bipolar fronto-occipital lead on one monkey, and a temporo-occipital lead on the other. The electrodes were chronically implanted stainless steel screws whose tips rested on the dura mater. The screw heads were wired to an electrical plug affixed to the skull with dental acrylic.

A strobe photo stimulator flashed 1/sec throughout the 1-hour data collection periods. The electroencephalogram was amplified 1.2×10^4 times, band-pass filtered at 0.8 Hz to 90 Hz, and analyzed by special purpose computer for visual evoked responses. Each individual VER was a composite of 100 EEG samples, and there were 12 to 18 VERs accumulated each data collection period. Filtered electroencephalogram was recorded on paper for visual analysis.

Nonparametric Friedman and Wilcoxon statistical analysis was utilized.[17] To be statistically significant the Friedman test had to show significant variation among the six blocks of data (D_0, D_2, D_4) from the control day and day of exposure, and the

Wilcoxon test between data blocks had to be significant in two directions, exposure data had to vary significantly from the control data of that day (e.g., D_0-D_2, exposure day), and exposure data had to vary significantly from its paired control block of the preceding day (e.g., D_2 control-D_2 exposure).

Three wave form amplitudes of the VER were analyzed (designated A, B and C waves). The A wave was positive at the occiput, had a latency of about 50 msec, and was measured base line to peak. The B wave was negative, had a latency of 80-120 msec, and was measured peak to valley. The C wave was positive, had a latency of 100 to 150 msec, and was measured valley to peak (Figure 2).

The Sidman avoidance task (free operant avoidance schedule) permitted the monkeys to avoid a 0.2-sec shock indefinitely as long as the interval between successive lever pulls did not exceed 10 sec. Lever tension was controlled by a solenoid and could be varied. A cue light directly above the lever remained on continuously for the 15 minutes in which the Sidman avoidance schedule was in effect. There was a 5-minute rest period (cue light off, no risk of shock) between sessions, and there were three Sidman sessions during each 1 hour data collection period. VERs were collected only during the Sidman sessions, not during rest sessions.

To ascertain the effect of other than neurotoxic variables on the VER two short experiments were conducted. In the first experiment the VER and Sidman avoidance data were collected for two 15-minute sessions in the normal manner. In the third session the lever tension was repeatedly changed, all other variables were left unchanged. Sidman and VER data from this session were then compared to the previous two sessions. In the second experiment control data were collected in the normal

manner for three 15-minute sessions. Halothane, an anesthetic gas, was then introduced into the chamber to a concentration of 0.15 percent (monkeys are usually anesthetized at about 1.5 percent halothane gas). Sidman and VER data from the next three 15-minute sessions were collected and compared to the three control sessions.

III. RESULTS

Sidman avoidance behavior was not affected at any PGDN vapor concentration tested, nor were there significant changes in the latencies of the VER wave forms or the amplitudes of the A and B waves.

However, more often than not, the amplitude of the C wave was affected. Figure 1 summarizes the data showing that at 2 ppm the C wave amplitude increased and

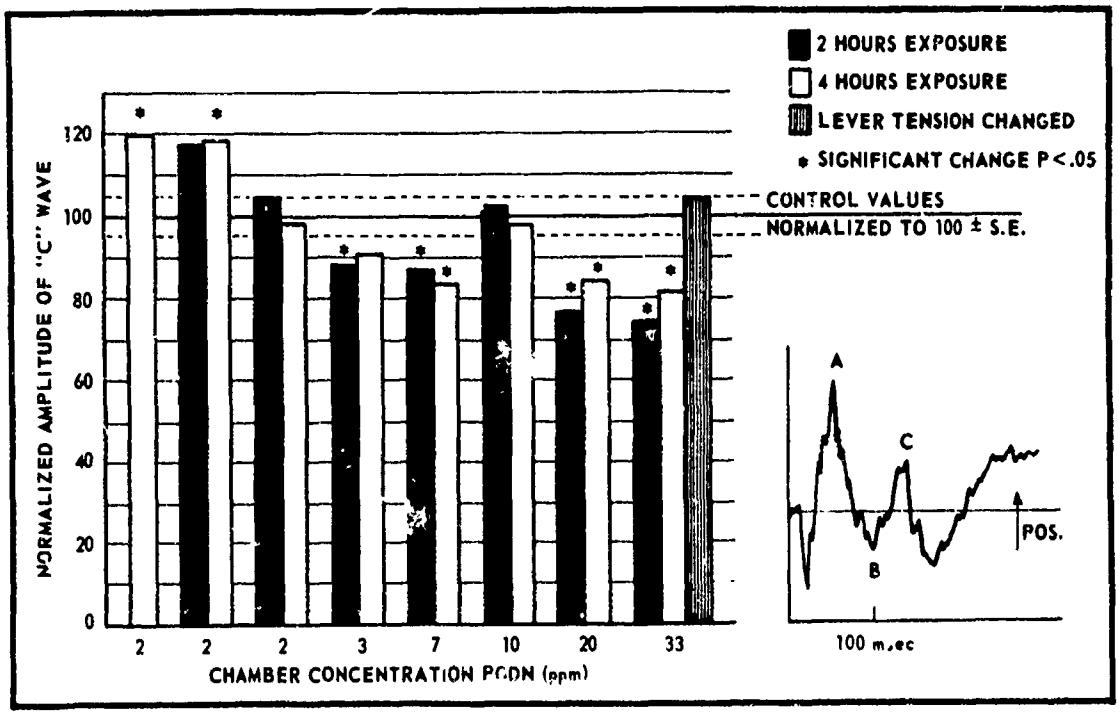

Figure 1. Effect of PGDN on VER C wave. Minimum of 1 week between exposures. Standard error (S.E.) was average of largest S.E. from each experiment. A and B waves were unaffected by PGDN.

at higher concentrations decreased. Note that once at 2 ppm, and at 10 ppm, there were no changes in any VER component. It can also be seen that after 4 hours exposure to 33 ppm, a simple change in the lever tension (the lever the monkey pulls in the Sidman task) caused a spontaneous return of C wave amplitude to control levels.

Figure 2 shows VERs at 0 ppm and after 4 hours at 33 ppm PGDN. Although the amplitude of the C wave is significantly depressed, it is readily apparent that the overall topography of the VER is unchanged.

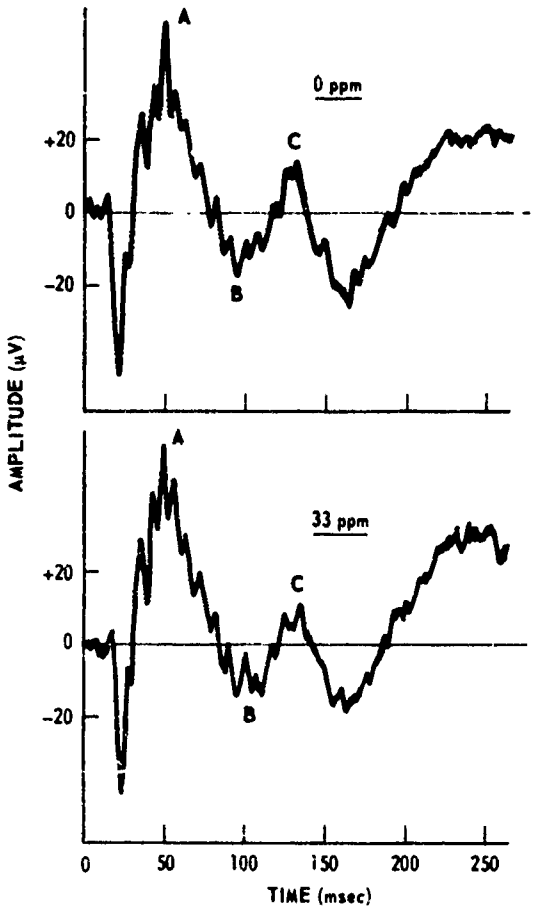

Figure 2. Rhesus monkeys VER at 0 ppm and after 4 hours at 33 ppm PGDN. Although the 130-msec positive wave (C wave) is depressed ($P < .05$), the general topography of the VER is unaltered.

During the course of training and stabilizing the monkeys' behavior it was found that the amplitude of the C wave could be increased or decreased nearly 40 percent by simple changes in the environment or routine. Intentional alteration of Sidman response lever tension caused a 30 percent increase in the C wave (Figure 3). No changes occurred in the A or B waves. On the other hand, exposure to a known neuropharmacologic agent, halothane, caused marked increases in A, B and C waves (Figure 4), but did not change Sidman avoidance behavior.

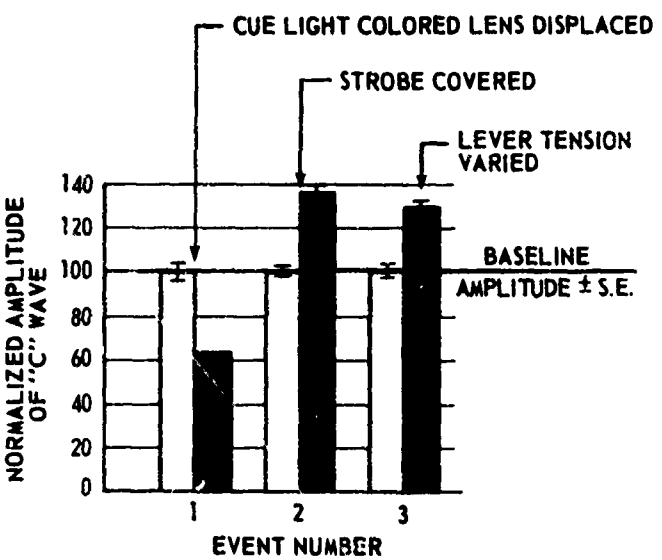

Figure 3. Effects of changed environment on VER C wave. A and B waves were unaffected. (1) During task performance the Sidman panel cue light lens came off. The black bar shows the amplitude of the C wave on the first VER after the lens was replaced. (2) Background "noise" was determined by collecting a VER with strobe covered. In this instance the strobe was prematurely covered, then uncovered for the last six VERs. The black bar shows the increased amplitude of the C wave of these last six VERs. (3) Control data were collected with uniform Sidman lever tension. The lever tension was solenoid controlled; lever tension was intentionally varied throughout the next 15-minute Sidman session, causing a stable 30 percent increase in the C wave.

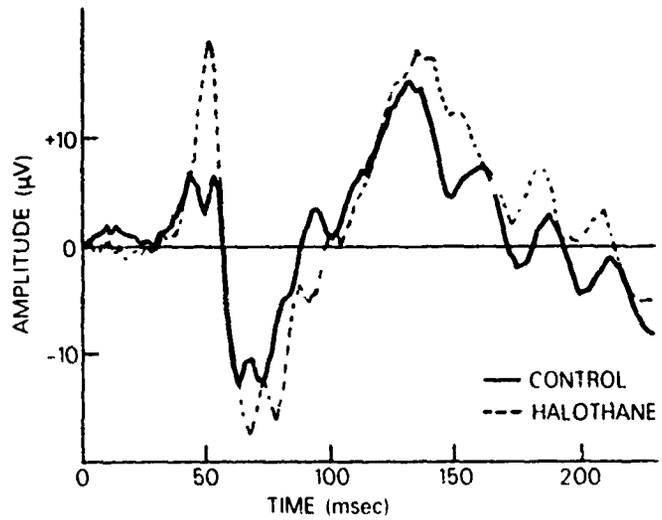

Figure 4. Effects of 0.15 percent halothane on VER latency and amplitude. At 2 hours postexposure all VER components had returned to normal.

IV. DISCUSSION

The direct visual pathway to the cortex is via the lateral geniculate, whereas nondirect pathways are routed through the superior colliculus and pretectal visual nuclei. Other connections of the superior colliculus are more general, such as the central grey substance, reticular formation and cerebellum. The visual system, through the central grey and midbrain reticular formation, contributes to the nonspecific thalamic projection nuclei which project to the cerebral cortex.[4] Destruction of the lateral geniculate abolishes early VER components, and destruction of the superior colliculus-pretectal area abolishes the late negative response.[14] The utility of the VER in toxicology is that the visual system interacts with this multitude of brain structures, and changes in the VER may occur when these structures are affected by toxic agents.

Accordingly, the visual evoked response has been used to study the CNS effects of numerous chemicals including PGDN,[18] carbon monoxide,[7] ethanol,[10,12] pentobarbital,[11] LSD and chlorpromazine,[1] and diazepam.[3,15] However, the interpretation of changes in the VER is always complicated because the shape of the wave form can be altered by three classes of variables; physical or stimulus events, physiological events, and psychological events.[19] Although psychological changes can occur at any latency of the VER, depending on location of the electrode, the most frequently reported changes occur at the late components.[13] Psychological events known to affect VERs include fear,[6] attention,[2] distraction,[2,13,19] anxiety,[19] and aversive stimuli.[9] To improve interpretability, the psychological variables need to be minimized and, if possible, separately identified.

The Sidman avoidance task was utilized in these PGDN experiments to minimize psychological variables. This task has no exteroceptive stimulus to warn the monkey of impending shock, and when shock occurs it is so brief that the animal does not terminate the shock, it postpones the next shock by pressing the lever. Successive lever presses can postpone shock indefinitely. The motivating drive state appears to be anxiety, and the avoidance behavior (lever pressing) is reinforced when it terminates or reduces the level of anxiety.[16] With time, Sidman avoidance behavior becomes quite stable and VER variability is markedly reduced. This small variability allows for a very sensitive indicator of neurotoxic and psychologic VER disruption.

The lack of effect of 33 ppm PGDN on the Sidman avoidance task is consistent with the observations of Young et al.[20] and extends the concentration range for no effect from 1.3 ppm to 33 ppm. It also adds the Sidman avoidance task to the other

behavioral tasks (visual discrimination and visual acuity threshold) that were not affected by high concentrations of PGDN.[8]

During exposure to PGDN vapors the monkeys' VERs changed only at the C wave, increasing 20 percent at 2 ppm and decreasing 25 percent at concentrations to 33 ppm. Following exposure to 33 ppm for 4 hours, the C wave amplitude returned to control values when the Sidman lever tension was altered. Since psychologic events such as change in environment or routine mainly affect later VER components (the C wave), the VER changes induced by PGDN could have been caused by its odorous, distracting properties, rather than a direct CNS effect. In contrast, exposure to halothane gas affected all VER components.

To separate psychologic from neurotoxic VER changes, PGDN concentrations in these experiments were increased to very high levels to alter the VER to such an extent that neurotoxic changes clearly dominated psychologic ones. This situation did not occur. Changes in VER occurred only at the C wave, and to a magnitude known to occur from psychologic events. Therefore interpretation of PGDN effects is ambiguous, even at 33 ppm. Interpretation of the human VER changes[18] is also difficult since the subjects could smell the vapor, had eye irritation, and had severe headaches.

Monkey VER data did not provide the hoped for continuity between human and nonhuman primate PGDN effects, and in view of the negative behavioral data in monkeys, questions still exist concerning the low concentrations of PGDN that affect man, and the high concentrations that affect monkeys. It may be that large species differences do, in fact, exist. Alternatively, the appropriate parameters may not have been chosen. Stewart et al.[18] reported that impairment in balance was manifest in humans

at 0.5 ppm; thus, balance would appear to be an appropriate and accessible parameter for future study in nonhuman primates.

REFERENCES

1. Buño, W., Jr., Villar, J. L., Tejerina, W. and García-Austt, E. Effect of LSD-25, chlorpromazine and metedrine upon visual evoked response in cats. Acta Neurol. Lat. Am. 16:64-73, 1970.

2. Cigánek, L. The effects of attention and distraction on the visual evoked potential in man: a preliminary report. Electroencephalogr. Clin. Neurophysiol. Supplement 26:70-73, 1967.

3. Ebe, M., Meier-Ewert, K.-H. and Broughton, R. Effects of intravenous diazepam (valium) upon evoked potentials of photosensitive epileptic and normal subjects. Electroencephalogr. Clin. Neurophysiol. 27:429-435, 1969.

4. Ford, D. H. and Schadé, J. P. Atlas of the Human Brain, pp. 43-44. New York, Elsevier Publishing Company, 1971.

5. Fultyn, R. V. Contaminant generators for continuous exposure inhalation chambers. Am. Ind. Hyg. Assoc. J. 22:49-53, 1961.

6. Hall, R. D. and Mark, R. G. Fear and the modification of acoustically evoked potentials during conditioning. J. Neurophysiol. 30:893-910, 1967.

7. Hosko, M. The effect of carbon monoxide on the visual evoked response in man. Arch. Environ. Health 21:171-180, 1970.

8. Jones, R. A., Strickland, J. A. and Siegel, J. Toxicity of propylene glycol 1,2-dinitrate in experimental animals. Toxicol. Appl. Pharmacol. 22:128-137, 1972.

9. Knispel, J. D. and Siegel, J. Tegmental stimulation: aversive effects on behavior and modulation of visual evoked potentials. Brain Res. 37:317-321, 1972.

10. Lewis, E. G., Dustman, R. E. and Beck, E. C. The effects of alcohol on visual and somato-sensory evoked responses. Electroencephalogr. Clin. Neurophysiol. 28:202-205, 1970.

11. Nakai, Y., Takeda, Y. and Takaori, S. Pharmacological studies on transmission in the central visual pathway in relation to effects of pentobarbital. Jap. J. Pharmacol. 21:721-730, 1971.

12. Nakai, Y., Takeda, Y. and Takaori, S. Effects of ethanol on afferent transmission in the central visual pathway of cats. Eur. J. Pharmacol. 21:318-322, 1973.

13. Regan, D. Evoked potentials and psychological variables. In: Evoked Potentials in Psychology, Sensory Physiology and Clinical Medicine, pp. 133-158. New York, Wiley Interscience, 1972.

14. Rose, G. Relationship of electrophysiological and behavioral indices of visual development in mammals. In: Brain Development and Behavior, Sterman, M., McGinty, D. and Adinolfi, A., editors, pp. 145-183. New York, Academic Press, 1971.

15. Sherwin, I. Differential action of diazepam on evoked cerebral responses. Electroencephalogr. Clin. Neurophysiol. 30:445-452, 1971.

16. Sidman, M. Avoidance behavior. In: Operant Behavior, Areas of Research and Application, Honig, W., editor. New York, Appleton-Century-Crofts, 1966.

17. Siegel, S. Nonparametric Statistics for the Behavioral Sciences. New York, N. Y., McGraw-Hill Book Company, Inc., 1956.

18. Stewart, R. D., Peterson, J. E., Newton, P. E., Hake, C. L., Hosko, M. J., Lebrun, A. J. and Lawton, G. M. Experimental human exposure to propylene glycol dinitrate. Toxicol. Appl. Pharmacol. 30:377-395, 1974.

19. Sutton, S. The specification of psychological variables in an average evoked potential experiment. In: Average Evoked Potentials, Methods, Results, and Evaluations, Donchin, E. and Lindsley, D., editors, pp. 237-297. National Aeronautics and Space Administration Report SP-191. Washington, D. C., U. S. Government Printing Office, 1969.

20. Young, R. W., Curran, C. R., Franz, C. G. and Jenkins, L. J . Jr. Effects of chronic inhalation of propylene glycol 1,2-dinitrate on the conditioned avoidance behavior of primates (in preparation).